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Clinical impact of myocardial mTORC1 activation in nonischemic dilated cardiomyopathy

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ABSTRACT

Background: Activity of mTOR complex 1 (mTORC1) has been shown to be up-regulated in animal models of heart failure. Here, we investigated the change and role of mTORC1 in human nonischemic dilated cardiomyopathy (NICM).
Methods: Endomyocardial biopsy specimens were obtained from patients with NICM ($n = 52$) and from Brugada syndrome patients with normal LVEF as controls ($n = 10$). The specimens were stained for phospho-ribosomal protein S6 (p-Rps6) and phospho-p70S6K (p-p70S6K), and the area with p-Rps6 signal was used as an index of mTORC1 activity. Using median mTORC1 activity, patients were divided into a high mTORC1 activity (H-mTOR) group and a low mTORC1 activity (L-mTOR) group.
Results: The ratio of p-Rps6-positive area in biopsy samples was 10-fold larger in patients with NICM than in controls ($2.0 \pm 2.2\%$ vs. $0.2 \pm 0.2\%$, $p < 0.01$). p-p70S6K signal level was higher in the H-mTOR group than in the L-mTOR group. The proportion of patients with a family history of cardiomyopathy was higher and the proportion of patients on ACE inhibitors or angiotensin receptor blockers was lower in the H-mTOR group than in the L-mTOR group. The p-Rps6-positive area was correlated with extent of myocardial fibrosis ($r = 0.46$, $p < 0.01$). The cardiac event-free survival rate during a 5-year follow-up period tended to be lower in the H-mTOR group than in the L-mTOR group (52.9% vs. 81.6%, $P = 0.10$).
Conclusion: Aberrant activation of mTORC1 in cardiomyocytes was associated with myocardial fibrosis and a trend for worse prognosis in patients with NICM, indicating that persistently activated mTORC1 contributes to progression of human heart failure.

1. Introduction

The mammalian/mechanistic target of rapamycin (mTOR) is a kinase regulating cell growth, proliferation, and survival [1]. mTOR exists as two mechanistically distinct complexes, mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is a master regulator of cell size and proliferation, and its activation suppresses autophagy. On the other hand, mTORC2 regulates activity of Akt and PKCα to control cell survival and polarity. Our recent study showed that mTORC2 plays a pivotal role in cytoprotective signaling activated by ischemic and pharmacological preconditioning [2]. mTORC1 is also involved in cardioprotective signaling against ischemia/reperfusion injury, though it may be dispensable for protection [2].

In contrast to its role in cytoprotection, the role of mTORC1 in heart failure has not been characterized fully. Genetic deletion of mTOR or raptor, an indispensable component of mTORC1, deteriorated cardiac function after transverse aortic constriction (TAC) [3,4]. However, pharmacological inhibition of mTORC1 activity by rapamycin or its analogs reversed myocardial remodeling and improved cardiac function in hearts with TAC-induced heart failure and in models of genetic cardiomyopathy [5,6,7]. These findings suggest that a physiological range of mTORC1 activity is required for maintenance of cardiac function under stress conditions, though persistently excessive activity of mTORC1 exerts a detrimental consequence to heart failure. However, our current knowledge regarding the role of mTORC1 in heart failure entirely relies on the results of animal experiments. It remains unclear whether mTORC1 activity is modified and affects the pathophysiology of human heart failure.

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The aim of this study was to clarify the clinical significance of mTORC1 activation in nonischemic dilated cardiomyopathy (NICM). To this end, we determined mTORC1 activity in endomyocardial biopsy specimens from NICM patients by immunohistochemical analysis and examined the relationships between the activity and clinical parameters.

2. Methods

Detailed Methods are in the Supplemental Material.

2.1. Subjects

We retrospectively analyzed data for 52 consecutive patients with NICM who underwent right ventricular endomyocardial biopsy from April 1998 to December 2012 (Supplemental Figure I). Ten patients with Brugada syndrome who had normal left ventricular ejection fraction (LVEF) served as controls. We defined cardiac event as a composite of cardiac death and admission for heart failure or arrhythmia.

2.2. Histological analysis of biopsy samples

Biopsy samples were fixed in 10% buffered formalin. Phosphorylation of ribosomal protein S6 (Rps6) and p70S6 kinase (p70S6K) being downstream of mTORC1 activation was assessed by immunostaining. The percentage of p-Rps6-positive area to total area in myocardial samples was calculated as an index of mTORC1 activity (Supplemental Figure II).

2.3. Statistical analysis

All data are presented as means ± SD. The difference was considered significant if the P value was <0.05.

3. Results

3.1. Localization of p-Rps6 signals in the human myocardium

Signals of p-Rps6 were detected in endothelial cells in both the control and NICM groups (Fig. 1A and B). Cardiomyocytes in the control group showed few p-Rps6 signals (Figs. 1A and B). In contrast, p-Rsp6-positive cardiomyocytes were frequently detected in the NICM group (Fig. 1C), particularly around fibrotic areas (Fig. 1D). Quantitative analysis showed that the ratio of p-Rsp6-positive area, an index of mTORC1 activity level, was 10-fold higher in the NICM group than in the control group (0.2 ± 0.2% vs. 2.0 ± 2.2%, $P < 0.01$, Fig. 1E). Little p-p70S6K signal was detected in the control group. In contrast, p-p70S6K signals were clearly observed in the nucleus and cytosol of the cardiomyocytes in the NICM group with high mTORC1 activity though the signal was weak in those with low mTORC1 activity (Supplemental Figure III).

3.2. Clinical characteristics of NICM patients with high mTORC1 activity

Using median mTORC1 activity (i.e., 1.21%), patients were classified into a high mTORC1 activity (H-mTOR) group and a low mTORC1 activity (L-mTOR) group as shown in Supplemental Figure II. There was no difference in age, gender, and concurrent metabolic diseases between the H-mTOR and L-mTOR groups (Table 1). Echocardiographic parameters and the proportions of patients with arrhythmia were comparable in the two groups. However, the proportion of patients who had a family history of cardiomyopathy was higher and the proportion of patients being treated with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) was lower in the H-mTOR group. The extent of myocardial fibrosis tended to be higher in the H-mTOR group than in L-mTOR group (Table 1) and was positively correlated with the level of mTORC1 activity (Fig. 1F).

3.3. Prognostic impact of mTORC1 activation in NICM

Eleven patients were transferred to other institutes after the biopsy, and 41 patients whom we could follow up for 5 years contributed to analysis of the relationship between mTORC1 activity and cardiac events. Patients who developed cardiac events had a larger p-Rsp6-positive area than that in patients who did not (1.3 ± 1.3% vs. 3.2 ± 3.1%, $P < 0.05$, Fig. 1G). The cardiac event-free survival rate tended to be lower in the H-mTOR group than in the L-mTOR group ($P = 0.10$, Fig. 1H), and the difference in survival rate was larger when H-mTOR and L-mTOR subgroups of patients under 60 years of age were compared ($P = 0.06$, Supplemental Figure IV).

4. Discussion

The present study showed that mTORC1 activity, which was assessed by phosphorylation of Rsp6 and p70S6K, was increased in patients with NICM and that the mTORC1 activity was closely correlated with myocardial fibrosis, a hallmark of end-stage heart failure (Fig. 1F). Interestingly, mTORC1 activity was higher in the ventricular myocardium of NICM patients in whom cardiac events later developed. Although no direct intervention on mTORC1 was performed in the present study subjects, we recently observed that heart failure in a case of NICM with high myocardial mTORC1 activity was significantly improved by treatment with everolimus, an mTORC1 inhibitor [8]. In addition, Hansen et al. [9] reported that the level of increased mTORC activity in skeletal muscle biopsy samples was significantly correlated with muscle weakness in patients with multiple sclerosis. Detrimental effects of mTORC1 upregulation on ventricular remodeling and dysfunction have been shown in animal models of pressure overload-induced heart failure and models of genetic cardiomyopathy [5,6,7,8], though a physiological level of mTORC1 activity is necessary to preserve cardiac function under pressure overload [3,4]. Taken together, the present findings support the notion that excess mTORC1 activity contributes to the development of heart failure and fibrosis in NICM. Whether mTORC1 activity is an independent predictor of cardiac events in NICM remains to be addressed by multivariate analysis of a larger number of study subjects.

The mechanism of mTORC1 upregulation in NICM was not clarified by this study, but a few possibilities can be speculated. Angiotensin receptor activation, induced by its ligands or pressure overload, might activate mTORC1. This possibility is indicated by data from *in vitro* experiments in which angiotensin receptor stimulation activated mTORC1 through β-arrestin-based signaling [10]. In fact, the proportion of patients being treated with ACEI/ARB was smaller in the H-mTOR group than in the L-mTOR group (Table 1). Accumulation of amino acids such as leucine in failing hearts [11] and genetic abnormality leading to mTORC1 activation [6,7,8] are also possible mechanisms of mTORC1 activation in NICM. We examined involvement of the PI3K/Akt pathway by immunostaining of p-GSK3β in *post-hoc* experiments. However, such an involvement was not supported by the finding that the area stained with p-GSK3β antibody was not correlated with the area stained with p-Rsp6 (data not shown).

Because mTORC1 plays multifunctional roles in the heart, ventricular dysfunction by up-regulation of mTORC1 activity in NICM is presumably mediated by multiple mechanisms. One such mechanism may be suppression of autophagic flux in cardiomyocytes. Accumulation of damaged or ubiquitinated proteins is a common feature of both failing hearts and hearts with disturbed autophahic flux [12,13]. The favorable effect of mTORC1 inhibitors on cardiac function was associated with promotion of autophagic flux [6,7]. Genetic deletion of atg5, which is necessary for autophagosome formation, exaggerated cardiac dysfunction after TAC [13]. Furthermore, we recently reported that everolimus,